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Abstract: Gram-positive bacteria are ubiquitous and diverse microorganisms that can survive and sometimes even thrive in continuously changing environments. The key to such resilience is the ability of members of a population to respond and adjust to dynamic conditions in the environment. In bacteria, such responses and adjustments are mediated, at least in part, through appropriate changes in the bacterial transcriptome in response to the conditions encountered. Resilience is important for bacterial survival in diverse, complex, and rapidly changing environments and requires coordinated networks that integrate individual, mechanistic responses to environmental cues to enable overall metabolic homeostasis. In many Gram-positive bacteria, a key transcriptional regulator of the response to changing environmental conditions is the alternative sigma factor (B) (B) has been characterized in a subset of Gram-positive bacteria, including the genera Bacillus, Listeria, and Staphylococcus Recent insight from next-generation-sequencing results indicates that (B)-dependent regulation of gene expression contributes to resilience, i.e., the coordination of complex networks responsive to environmental changes. This review explores contributions of (B) to resilience in Bacillus, Listeria, and Staphylococcus and illustrates recently described regulatory functions of (B).

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MINIREVIEW

Resilience in the Face of Uncertainty: Sigma Factor B Fine-Tunes Gene Expression To Support Homeostasis in Gram-Positive Bacteria

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Gram-positive bacteria are ubiquitous and diverse microorganisms that can survive and sometimes even thrive in continuously changing environments. The key to such resilience is the ability of members of a population to respond and adjust to dynamic conditions in the environment. In bacteria, such responses and adjustments are mediated, at least in part, through appropriate changes in the bacterial transcriptome in response to the conditions encountered. Resilience is important for bacterial survival in diverse, complex, and rapidly changing environments and requires coordinated networks that integrate individual, mechanistic responses to environmental cues to enable overall metabolic homeostasis. In many Gram-positive bacteria, a key transcriptional regulator of the response to changing environmental conditions is the alternative sigma factor σ^B. σ^B has been characterized in a subset of Gram-positive bacteria, including the genera *Bacillus*, *Listeria*, and *Staphylococcus*. Recent insight from next-generation-sequencing results indicates that σ^B-dependent regulation of gene expression contributes to resilience, i.e., the coordination of complex networks responsive to environmental changes. This review explores contributions of σ^B to resilience in *Bacillus*, *Listeria*, and *Staphylococcus* and illustrates recently described regulatory functions of σ^B.

Gram-positive bacteria can thrive in a myriad of environments, ranging from water, soils, and food surfaces to different types of hosts. One key to their survival lies in their ability to respond efficiently to different and rapidly changing environments by shaping their transcriptome in response to environmental conditions. A key regulator contributing to the survival of multiple Gram-positive genera under changing conditions is the alternative sigma factor σ^B, a subunit of the RNA polymerase holoenzyme. σ^B is well established as contributing to the response and survival of *Bacillus*, *Listeria*, and *Staphylococcus* species during exposure to a variety of adverse conditions, such as low pH, bile, and osmotic stress (1–7). One example of a mechanistically simple σ^B-mediated stress response is the σ^B-dependent expression of *bsh*, the gene encoding bile salt hydrolase in *Listeria monocytogenes*, upon encountering bile in the host digestive tract (8). σ^B also plays a clear role in *L. monocytogenes*' acid response; σ^B is activated in response to many types of acid stress (e.g., exposure to acidic pH of 2.5 to 4.5) (9) and directly upregulates the transcription of many genes that encode effector proteins that counteract acid stress (10–14). In this review, we contrast a simple, mechanistic definition of stress response, such as that mediated by increased expression of bile salt hydrolase, with the concept of resilience, which includes but is not limited to stress response. Resilience is defined by the Merriam-Webster dictionary as "an ability to recover from or adjust easily to misfortune or change." Resilience, therefore, requires not only a successful response to an initial change or stress but also the ability of an organism to continue to respond to or to take advantage of subsequent changes in the environmental conditions. For pathogens, the concept of resilience includes the production of the virulence factors needed to mediate survival within hosts, e.g., for evasion of the host immune system and the ability to obtain nutrients from the host environment.

The enteric pathogen *Listeria monocytogenes* offers remarkable examples of σ^B-mediated responses to the complex and rapidly changing environmental conditions encountered in animal hosts, which provide this pathogen with the resilience capacity to cause human infection. To illustrate, bacterial exposure to an initial stress condition (e.g., acidic pH in the stomach) not only triggers the expression of acid resistance functions that facilitate survival in this environment but also upregulates the transcription of genes important for survival in subsequent host compartments. For example, the σ^B-mediated response to low pH also enables the invasion of intestinal epithelial cells, which is partially but not solely facilitated by σ^B-dependent contributions to the transcription of invasion proteins (e.g., *L. monocytogenes* InlA) (15, 16). Thus, σ^B-dependent contributions to resilience go beyond simple upregulation of a specific stress response gene. This review summarizes recent insights into the σ^B regulon in *Bacillus*, *Listeria*, and *Staphylococcus* species, including the complex σ^B-mediated regulatory network interactions that facilitate the integrated and coordinated fine-tuning of physiological functions important for bacterial resilience. Importantly, all three genera include environmentally transmitted and foodborne pathogens, which require the ability to adapt, survive, and grow in diverse and rapidly changing environments for successful transmission to and from their animal hosts.

THE ALTERNATIVE SIGMA FACTOR σ^B

Sigma factors are required for transcription initiation, as they confer promoter specificity to the RNA polymerase holoenzyme and induce helix destabilization to expose the DNA template strand for RNA synthesis. A primary sigma factor, σ^A, in Gram-positive

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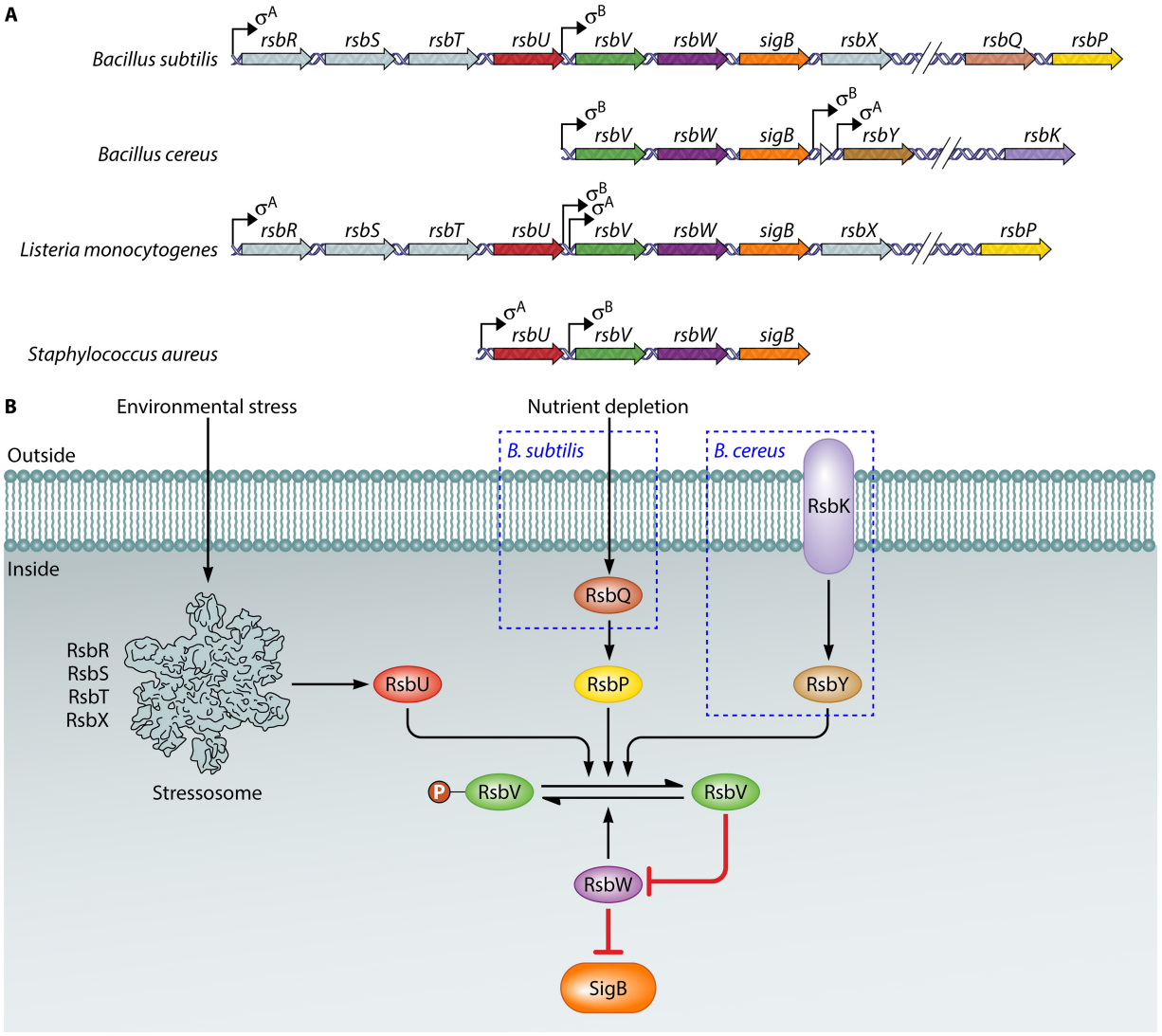

FIG 1 Regulation of SigB expression and activity. (A) Conservation of *sigB* and *rsb* genes and operon structures across Gram-positive bacteria. Filled arrows represent open reading frames color coded by homology. Thin bent arrows indicate promoters, and the respective sigma factors are shown. Sequence, organization, conservation, and promoter information was obtained from NCBI (http://www.ncbi.nlm.nih.gov/), BioCyc (http://biocyc.org/), and STRING (http://string-db.org/). (B) Signal transduction leading to SigB activation. See the text for details.

bacteria (17) is required for housekeeping functions essential for cellular growth and reproduction. In general, bacteria possess at least one alternative sigma factor (e.g., σ^B) to mediate specialized functions, such as cell differentiation, biofilm formation, and modulation of an appropriate response to changing environmental conditions (18).

σ^B is one of the most comprehensively studied alternative sigma factors of Gram-positive bacteria. This sigma factor is found in a subset of Gram-positive bacteria and has been identified in *Bacillus*, *Listeria*, and *Staphylococcus* species, as well as in other genera in the order *Bacillales* (e.g., *Oceanobacillus* and *Paenibacillus*) (19–21). σ^B was first identified in 1980 in the Gram-positive model organism *Bacillus subtilis* (22). While the specific function of σ^B in *B. subtilis* was initially unknown, subsequent work showed that this alternative sigma factor plays a key role in bacterial survival under adverse conditions, including entry into stationary phase (3–6). In the late 1990s, σ^B was also identified in *L. monocytogenes*, as well as in other *Listeria* species (1) and in *Staphylococcus* species (23). The specific functions initially described for σ^B in *L. monocytogenes* included bacterial responses to acid (1) and osmotic stress (2). Similarly, the roles of σ^B originally described in *Staphylococcus aureus* involved heat, acid, and hydrogen peroxide resistance (7).

σ^B activity is tightly controlled both transcriptionally and post-transcriptionally. The multiple levels of regulation allow the bacterial cell to rapidly induce σ^B activity in response to different environmental conditions. Interestingly, the systems regulating σ^B differ considerably among *Staphylococcus*, *Listeria*, and *Bacillus* species (Fig. 1). Both *B. subtilis* (as well as *Bacillus licheniformis*, *Bacillus halodurans*, *Bacillus clausii*, and *Oceanobacillus iheyensis*) and *L. monocytogenes* (as well as *Listeria innocua* and *Listeria welshimeri*) possess an 8-gene cluster that includes the *rsbVW-sigB-rsbX* operon, regulated by a σ^B-dependent promoter, and an upstream *rsbRSTU* operon, which is transcribed from a σ^A-de-

TABLE 1 Overview of σ^B functions in *B. subtilis*, *L. monocytogenes*, and *S. aureus*

Organism	Size of σ^B regulon (references)	Associated function(s) (reference[s])
B. subtilis	~150 genes (19, 47, 48, 165)	Sporulation (49) Antibiotic resistance (61, 166) Growth and starvation (167) Transitional growth phase (168)
L. monocytogenes	~130 genes (8, 72, 162)	Osmotic, cold, and acid stress (10, 11, 50, 70, 169) Virulence (8, 15, 16) Host cell invasion (15) Bile resistance (170) Attachment community formation (98) Antibiotic resistance (62)
S. aureus	~200 genes (51–54)	Antibiotic resistance (58, 171–174) Virulence (175) Cell envelope homeostasis (89) Persistence (176) Biofilm (177) Host cell internalization (148) Intermediate metabolism (52) Membrane transport processes (52)

pendent promoter (1, 24–26). Interestingly, *Bacillus cereus* (as well as *Bacillus anthracis* and *Bacillus thuringiensis*) only has a 4-gene *rsbVW-sigB-rsbY* operon, which includes separate σ^B promoters upstream from each of the genes *rsbV* and *rsbY*, as well as an additional σ^A promoter upstream from *rsbY* (Fig. 1A) (27–29). *S. aureus* lacks *rsbRST* as well as *rsbX*; the resulting *rsbUVW-sigB* operon includes a σ^A promoter upstream from *rsbU* and a σ^B promoter upstream from *rsbV* (30). Despite the differences in operon structure, all of these organisms incorporate a σ^B-dependent positive-feedback loop that regulates the transcription of the *sigB* and *rsb* genes.

Posttranscriptional regulation of σ^B activity involves a phosphorylation cascade that is catalyzed by the regulation of sigma B (Rsb) proteins. During exponential growth, σ^B is held in an inactive state, bound to the anti-sigma factor RsbW. The activation of σ^B requires binding of the dephosphorylated form of the anti-anti-sigma factor RsbV to anti-sigma factor RsbW, which then releases σ^B, thereby allowing σ^B to bind to RNA polymerase and, thus, to trigger transcription at cognate promoters (Fig. 1B) (19, 31). The control of σ^B activity by RsbVW is highly conserved in species containing σ^B (26); however, the upstream regulation of this sigma/anti-sigma/anti-anti-sigma partner-switching module differs among genera. In *B. subtilis* and *L. monocytogenes*, the phosphatases RsbP and RsbU dephosphorylate RsbV in response to energy stress (32–34) and environmental stress (35–40), respectively (reviewed in reference 20; 34, 41). RsbU activity is controlled by the switch kinase RsbT and its antagonist RsbS. Together with RsbR and RsbS, RsbT is part of the "stressosome," a multiprotein signaling hub that includes multiple copies of each protein (reviewed in reference 42). In contrast to *B. subtilis*, posttranscriptional regulation of RsbVW-σ^B in *B. cereus* involves the RsbK multisensory histidine kinase and the RsbY phosphatase (43). Similarly, *S. aureus* also lacks RsbRST, and RsbU appears to play a different role than in *B. subilis* (44, 45). Importantly, posttranslational activation of σ^B, which is conserved across organisms, allows for very rapid response of bacterial cells to changes in environmental conditions (induction of σ^B activity takes <5 min) (3, 46), which is critical for resilience under rapidly changing environmental conditions.

DIVERSE ROLES OF σ^B IN BACTERIAL RESILIENCE IN *LISTERIA*, *STAPHYLOCOCCUS*, AND *BACILLUS* SPECIES

The number of genes regulated by σ^B varies between species (Table 1). The σ^B regulon in *B. subtilis* has been reported to include between 150 and 200 genes, depending on the experimental conditions used in a given study (19, 44, 47, 48). Some of the key roles of σ^B in *Bacillus* species include resistance to heat, acid, starvation, nitric oxide, and osmotic stress and antibiotics, and σ^B is also involved in integrating the stress adaptation and sporulation pathways (49). Key stress response functions regulated by σ^B in *Listeria* species include the responses to acid, oxidative, and energy stress (1, 10, 50). Overall, σ^B appears to upregulate ~200 genes in *L. monocytogenes* (51–54). The σ^B regulon in *S. aureus* appears to encompass about 200 genes (52–55), the products of which, in addition to general stress response, are also involved in resistance to several clinically important antibiotics, such as methicillin and vancomycin (55–58).

Despite a number of conserved functions and features of σ^B-dependent regulatory systems, the specific functions regulated by σ^B differ considerably among species. One hypothesis drawn from these observations is that the roles of σ^B and the σ^B regulon may have evolved independently to facilitate bacterial survival under conditions encountered in specific environments. For example, σ^B facilitates survival of acid stress in *Bacillus* and *Listeria* species (reviewed in references 19 and 31), whereas the contributions of σ^B to acid stress resistance appear to be minimal in *S. aureus* (59). Osmotic stress induces σ^B activity in both *Listeria* and *Bacillus* species (13, 60), and σ^B plays a role in antibiotic resistance for *Bacillus*, *Listeria*, and *Staphylococcus* species (58, 61, 62). Interestingly, in *B. subtilis*, the σ^B regulon plays a role in the response to nitric oxide (NO). While the flavohemoglobin encoded by *hmp* is the main detoxifier of NO (63), a study using reporter fusions to a σ^B-dependent promoter showed that σ^B activity increased upon nitric oxide stress under aerobic conditions in *B. subtilis* strain PB198 (64). Interestingly, depending on the source of the nitrous stress, the pathway by which σ^B was activated differed: NO gas induced σ^B activity via RsbP and the energy stress pathway, while sodium nitroprusside as a NO donor induced σ^B activity via RsbU

and the environmental-stress-dependent pathway (64). The results of a study comparing the responses to the NO donor MAHMA-NONOate at the protein level in *B. subtilis* and *S. aureus* confirmed the induction of the σ^B regulon by NO in *B. subtilis* but not in *S. aureus* (44). This is not surprising, since *S. aureus* lacks the RsbP-dependent regulatory pathway to induce σ^B activity (52).

σ^B clearly plays a broad and diverse role in regulating gene expression among different Gram-positive genera. Multiple lines of evidence indicate that σ^B is involved in many functions beyond the regulation of individual genes that facilitate survival of a specific stress. In the following sections, we highlight these broader functions by illustrating recent findings of the involvement of σ^B in bacterial resilience, including σ^B-dependent contributions to (i) metabolism, (ii) cell envelope homeostasis, (iii) biofilms, and (iv) pathogenesis.

σ^B IN METABOLISM OF HARMFUL COMPONENTS AND UTILIZATION OF DIFFERENT CARBON SOURCES

Increasing evidence supports the idea that σ^B contributes to the regulation of metabolic functions, including (i) metabolism of harmful components to allow survival and (ii) adaptation to allow the utilization of different carbon sources (19, 31, 65), as detailed below. Both of these metabolic functions clearly contribute to bacterial resilience, as the abilities to counteract harmful compounds and to nimbly adapt the cell's metabolism to changing energy sources are critical for the growth and survival of bacteria exposed to rapidly changing and complex environments.

One example of σ^B's contributions to bacterial survival in the presence of harmful components is the σ^B-mediated expression of *bsh*, which encodes a bile salt hydrolase, in *L. monocytogenes* (8). The expression of bile salt hydrolase allows *L. monocytogenes* to survive bile encountered in the small intestine. For a foodborne pathogen, exposure to bile occurs shortly after bacterial passage from the stomach to the duodenum. We speculate that the activation of σ^B in the acidic environment of the stomach prepares *L. monocytogenes* for subsequent survival in the hostile environment of the small intestine, which not only contains bile but also represents a hyperosmotic environment (66, 67). Another example of σ^B-mediated metabolic capabilities relevant to resilience is the consumption of protons by decarboxylation of glutamate to γ-aminobutyrate (GABA) (GAD system), which helps to elevate the intracellular pH and, hence, facilitates bacterial survival in acidic environments; the expression of *L. monocytogenes gad* genes is, in part, σ^B dependent (68–70).

Increasing evidence also indicates that *L. monocytogenes* σ^B is involved in complex networks regulating carbohydrate metabolism-related functions, including phosphotransferase systems (PTS) and the metabolism of GlcNAc or glycerol. Oliver et al. identified a σ^B-dependent promoter upstream from the *mpo* operon (*mpoABCD*) that encodes a mannose-specific PTS (71). Conversely, a proteomics study showed that σ^B negatively regulates other PTS components, including Lmo1997, Lmo1998, Lmo2002, Lmo0427, Lmo0484, and Lmo2648 (72). A recent study by Wang et al. (73) that used deletion mutants to assess the growth of *L. monocytogenes* in medium supplied solely with PTS-dependent carbon sources found that growth under these conditions was dependent on coregulation by σ^B and two other alternative sigma factors, σ^L and σ^H, in a complex regulatory network that was influenced by temperature and the respective carbon source (glucose, mannose, cellobiose, or glycerol) (74). Importantly, PTS-mediated carbohydrate uptake is also linked to virulence (75, 76). A number of studies have shown that when PTS systems are active, positive regulatory factor A (PrfA), the predominant transcriptional regulator of virulence genes, is downregulated via several intermediate steps that are not yet fully resolved (77). The currently proposed model involves PTS^{Mpo}, PTS^{Man}, and the activator of the *man* operon, ManR. Glucose uptake through PTS^{Mpo} activates ManR by dephosphorylation, which in turn upregulates the transcription of the *manLMN* operon encoding PTS^{Man} (78, 79). Uptake of glucose through PTS^{Man} then results in PrfA inhibition via a mechanism that appears to involve dephosphorylation of the $EIIAB^{Man}$ subunit (77, 78). These data not only indicate an involvement of σ^B in *L. monocytogenes* carbohydrate metabolism but also link this involvement with regulation of PrfA activity and, therefore, virulence. *L. monocytogenes* σ^B also regulates an operon involved in glycerol metabolism (80); specifically, proteomics data indicate that σ^B upregulates three proteins (Lmo2695 to Lmo2697) that appear to be subunits of dihydroxyacetone kinase, which is part of the glycerol metabolism pathway. The identification of a σ^B promoter consensus sequence upstream from lmo2695 and observation of the reduced ability of an *L. monocytogenes sigB* mutant to use glycerol as a sole carbon source further support σ^B's contributions to the regulation of glycerol metabolism (80). Glycerol is used as a non-PTS-dependent alternative carbon source by intracellular *L. monocytogenes* bacteria. Upregulating glycerol metabolism also upregulates PrfA activity, again highlighting a link between carbohydrate metabolism and virulence functions (81).

L. monocytogenes σ^B also regulates the transcription of the *nagABR* operon (13, 72), which encodes two deaminases necessary for *N*-acetylglucosamine (GlcNAc) degradation (NagA and NagB) (82) and NagR, which functions as a transcriptional inhibitor in the absence of GlcNAc (83). Apart from being a vital part of the bacterial cell wall, monomeric GlcNAc is also a major component of chitin; therefore, GlcNAc is among the most abundant carbon sources in the environment. *L. monocytogenes* metabolizes GlcNAc in a temperature-dependent fashion, but only in the absence of glucose catabolites (84). σ^B-dependent regulation of GlcNAc catabolism thus represents another example of a situation where σ^B contributes to bacterial resilience by facilitating the use of alternative carbohydrate sources in the absence of glucose. The mechanism of catabolite repression that appears to be involved in this regulatory circuit remains to be elucidated.

In *S. aureus*, σ^B appears to contribute indirectly to the regulation of hyaluronidase, which hydrolyzes hyaluronic acid. Hyaluronic acid is a component of the extracellular matrix present in many tissues that can be used as a carbon source by *S. aureus* and many other bacteria (85). The transcription of *S. aureus* hyaluronidase, encoded by *hysA*, is controlled by the accessory gene regulator (Agr) quorum-sensing system (86, 87). Work by Ibberson et al. (88) suggests that σ^B (as well as CodY) downregulates *agr* and therefore indirectly and negatively controls hyaluronidase activity in *S. aureus*. Based on the observation that *sigB* and *codY* mutants express higher levels of hyaluronidase activity, *hysA* is proposed to be under direct positive control by the effectors of Agr and negatively modulated by CodY and σ^B (88). The specific mechanisms affecting *hysA* transcription levels by altering the balance among Agr, CodY, and σ^B and the question of whether these mechanisms

contribute to virulence by providing access to a readily available host carbon source remain to be elucidated.

σ^B IN CELL ENVELOPE HOMEOSTASIS

The cell wall ensures bacterial integrity by maintaining a physical barrier between the cell and its environment and by giving it its shape. Maintenance of cell wall homeostasis is an important factor for bacterial resilience during growth and adaptation to changing conditions. σ^B-dependent gene regulation contributes to such resilience in various ways, including (i) cell envelope homeostasis and modification of cell envelope composition in the absence of stress (12, 89) and (ii) translation of stress signals at the cell envelope into upregulation of virulence factors (90). This section concentrates on *L. monocytogenes* and *S. aureus*, as there are no clear data on contributions of σ^B to cell wall homeostasis in *Bacillus* species.

In *L. monocytogenes*, via a σ^B-dependent promoter, σ^B positively regulates the transcription of *dapE* (71), which encodes a key intermediate (mesodiaminopimelate) of the peptidoglycan synthesis pathway (80). DapE is upregulated in stationary-phase bacteria (91) and within host cells and is speculated to mediate the anchoring of surface proteins to the cell wall (92). Abram et al. (12) reported that a *sigB* mutant showed unusual Gram-staining properties, even in the absence of stress, supporting σ^B-dependent regulation of bacterial cell wall components. In *S. aureus*, σ^B plays a role in cell wall homeostasis via regulation of *asp23* expression. Asp23 has been used in many *S. aureus* studies as a marker for σ^B activity because its transcription is exclusively regulated by σ^B (93). It is also one of the most abundant proteins in stationary-phase *S. aureus*, but until recently, its function remained unclear. Muller et al. (89) showed that Asp23 is anchored to the cell wall by AmaP and that improper localization or deletion of Asp23 results in the upregulation of cell wall stress genes. These findings suggest that Asp23 plays a role in cell envelope homeostasis in stationary-phase cells and that the disruption of either the production of Asp23 or its correct localization results in increased stress for the cell. A recent study by Ishii et al. (90) also indicated that the response to environmental stress sensed at the *S. aureus* cell wall results in σ^B-dependent upregulation of virulence genes. They used RNA-seq to explore the transcriptomic change in *S. aureus* strain Newman in response to exposure to surfactant, which would be one of the first hurdles encountered by *S. aureus* upon reaching the lung. According to the Centers for Disease Control and Prevention (CDC), lung infections by *S. aureus* occur mainly in polymorbid patients and account for 13 to 15% of clinical methicillin-resistant *Staphylococcus aureus* (MRSA) infections (http://www.cdc.gov/abcs/reports-findings/survreports/mrsa12.pdf). Three key genes were upregulated in *S. aureus* exposed to surfactant, including *essC* (encoding a type VII secretion system) (94), *hlgB* (encoding hemolysin gamma, which induces pores in target cells) (95), and *psiA* (encoding a protein with similarity to proteins involved in lipid metabolism). This unique response to surfactant as a stimulus was found to be σ^B dependent, as the mRNA levels for *essC*, *hlgB*, and *psiA* did not increase in response to surfactant in a *sigB* mutant strain. In contrast, none of the general inducers of virulence genes in *S. aureus* (Agr, ArlS, and Sae) were necessary to elicit the observed regulatory response to surfactant. Mouse infection experiments also showed that mutations in *essC*, *hlgB*, and *psiA* resulted in reduced numbers of bacteria recovered from the lungs and a lower death rate, further supporting a role for σ^B in the production of cell wall components (EssC) and lipid metabolism (PsiA) with a proposed link to virulence. These data provide an example of σ^B-dependent regulation both of cell wall-associated functions and of proteins with potential virulence-related functions that facilitate resilience of *S. aureus* in a hostile host-associated environment.

σ^B IN BIOFILMS

The formation of either true biofilms with a typical extracellular matrix or bacterial attachment communities (which do not show the accumulation of extracellular matrix typical for traditional biofilms) facilitates bacterial survival in a variety of different environments, such as in food processing facilities or clinical settings, including on foreign objects that may be placed in a patient for extended periods of time (e.g., catheters and implants). As such, biofilms represent resilient bacterial communities. Increasing evidence indicates that σ^B may play a role in biofilm formation or the establishment of attachment communities in *Bacillus*, *Listeria*, and *Staphylococcus* species (96–98).

While the main transcriptional regulator for *B. subtilis* biofilms is Spo0A (99), σ^B also appears to contribute specific regulatory functions to biofilm formation in this organism. The role of Spo0A-dependent regulation in *B. subtilis* is complex, and the control of Spo0A levels is not yet completely understood (for a review, see reference 100). Briefly, intermediate levels of Spo0A induce the production of a biofilm matrix via a signaling cascade of repressors and anti-repressors, including SinI, SinR, SlrR, and AbrB, while high levels of Spo0A accumulate in some cells during biofilm maturation and induce sporulation and dispersion from the mature biofilm. Spo0A is active in its phosphorylated form, and the phosphatase Spo0E regulates Spo0A activity by dephosphorylating Spo0A (101). Reder et al. identified a σ^B-dependent promoter upstream from *spo0E in silico* and experimentally showed σ^B-dependent transcription of *spo0E* (49). A *sigB* mutation in *B. subtilis* strain 168 severely affected biofilm formation and morphology, a phenotype that was shown by Nagorska et al. (96) to involve σ^B-dependent expression of the putative exopolysaccharide (EPS) synthetase *yxaB*. Using quantitative reverse transcription (qRT)-PCR, *yxaB* was determined to be transcribed in an operon with *yxaA*; *in silico* analysis and experiments using reporter fusions identified a σ^B-dependent promoter upstream from the transcriptional start site of *yxaA*. The biofilm-deficient phenotype of the *sigB* mutant could be partially rescued by overexpression of *yxaB*, which resulted in structured, floating biofilms that were more fragile than the biofilms formed by the parent strain (96).

In *L. monocytogenes*, many aspects of biofilm formation still remain to be explored (102). A number of studies show that *L. monocytogenes* has the ability to rapidly and strongly adhere to inanimate surfaces (103, 104). However, attempts to unambiguously show that *L. monocytogenes* forms true biofilms with the typical accumulation of extracellular matrix find either a lack thereof (105, 106), a very sparse extracellular matrix (107, 108) with strain-dependent formation of extracellular fibrils of unclear function (109), or ball-shaped structures held together by chains of interconnected cells (110). To date, the exact nature of the fibrils in *L. monocytogenes* biofilms remains to be determined. A study that observed filaments in biofilms formed by certain strains of *L. monocytogenes* argued that these filaments were probably flagella, because they were not observed in nonmotile strains of *L.*

monocytogenes (111). Some studies (98, 112) suggest that σ^B plays a role in establishing *Listeria* attachment communities. For example, van der Veen and Abee (98) showed differential *sigB* expression in *L. monocytogenes* strain EGD-e during growth on surfaces. While posttranscriptional regulation of σ^B activity may inhibit a proportional increase in σ^B activity in parallel with increased *sigB* transcript levels, higher *sigB* transcript levels were found in attachment communities on polystyrene in static medium than in planktonic cells grown in broth, a finding that was even more pronounced under continuous-flow conditions. The cell density of attachment communities on surfaces was significantly lower for a *sigB* null mutant than for a wild-type strain (98) in both static and continuous flow systems, further supporting a role for σ^B in *Listeria* attachment to surfaces. In another study, no difference in adherence of the cells to stainless steel surfaces was observed between an *L. monocytogenes* 10403S *sigB* null mutant and a wild-type strain (113), suggesting that the involvement of σ^B in the formation of attachment communities and biofilms may be strain specific and/or may depend on environmental conditions.

In *S. aureus*, a recent study found reduced biofilm formation in *sigB* and *sarA* mutants (as well as in *atl*, *codY*, and *rsbU* mutants), with the *sarA* mutation having the largest effect; as *sarA* is positively regulated by σ^B, the effect of σ^B on biofilm formation may be due to reduced *sarA* transcription in the *sigB* null mutant (114). The same study suggested that impaired biofilm production in *sigB* and *sarA* mutants is linked to increased production of extracellular proteases that may degrade the biofilm matrix in these strains. Negative regulatory effects of σ^B and SarA on the production of extracellular proteases (115) are supported by the fact that the biofilm-impaired phenotype of *sigB* and *sarA* mutants is further reduced in a protease-deficient *S. aureus* strain compared to the biofilm formation by strains with the same mutations in a wild-type background (114).

Interestingly, the effects of a *sigB* null mutation on biofilm formation were influenced by the culture conditions: the addition of human plasma to the growth substrate reversed the *sigB* mutation phenotype, reflecting the complexity of the regulatory network that fine-tunes biofilm regulation. Additional circumstantial evidence for σ^B involvement in *S. aureus* biofilm formation has been reported (97); reduced biofilm generation was observed in naturally occurring *S. aureus* "white variants," which carry mutations in their *sigB* coding sequence. However, no experimental data were presented to exclude the possibility that the biofilm formation defect in the white variant strains is due to mutations in genes other than *sigB*. A possible mechanistic explanation for the reduced cell mass in biofilms formed by the white variant strains is that *sigB* mutations may relieve the inhibitory effect of σ^B on the Agr regulon, which occurs in the wild type (116, 117). Increased AgR activity, in turn, upregulates extracellular nucleases, proteases, and hemolysins that degrade the extracellular matrix of the biofilm.

A recent study showed that the *S. aureus* hyaluronidase HysA regulates the amount of host hyaluronic acid that is incorporated into biofilms *in vivo*. *hysA* in turn is under indirect negative control by σ^B (118). Interestingly, several studies support a role for σ^B in the biofilm formation of *Staphylococcus epidermidis*; mutations in *sigB* or *rsbU* lead to reduced biofilm production (119, 120), and the stability of biofilms under nutrient limitation is σ^B dependent (121). σ^B-dependent biofilm regulation involves the *ica* operon in an oxygen-dependent way (122, 123). *ica* encodes the polysaccharide intercellular adhesin (PIA) that is crucial for intercellular adhesion during biofilm formation. In the model proposed by Knobloch et al. (120), *ica* transcription is downregulated by IcaR, which is inhibited in turn by σ^B, resulting in a net positive effect of σ^B on PIA production. This conclusion is supported by the reduced biofilm formation phenotype of *sigB* mutant strains and by the results from Northern blot analyses of *ica* and *icaR* transcript levels in various strains: in cells with an intact σ^B operon, *icaR* transcript levels are low while *ica* transcript levels increase, and the inverse effects occur in the absence of active σ^B (120).

σ^B IN PATHOGENESIS

Perhaps the most illuminating examples that illustrate the contributions of σ^B to resilience are the roles of this alternative sigma factor in pathogenesis, virulence, and survival in the rapidly changing environments encountered in the host. Specifically, for foodborne and enteric pathogens, the ability to survive the changing conditions encountered along the gastrointestinal tract is essential to host invasion. Bacterial responses to changes in pH, temperature, and bile and salt concentrations are core functions of σ^B, as discussed above. In addition, there is clear evidence that σ^B also regulates functions directly relevant for pathogenesis and the coordinated expression of virulence genes in the host. While preliminary data on the σ^B regulon in the pathogenic *Bacillus anthracis* suggested that a *B. anthracis sigB* null mutant may be less virulent in a mouse model (124), further studies on the contributions of σ^B to virulence in pathogenic *Bacillus* species are still needed. Hence, the section below focuses on *Listeria* and *Staphylococcus*.

While PrfA is a key transcriptional regulator of the core virulence genes in *L. monocytogenes*, σ^B contributes to the larger network that regulates virulence factors; specifically, a number of studies have shown considerable and complex overlaps between the σ^B and PrfA regulons (8, 13, 125, 126), including direct transcriptional regulation of genes by both σ^B and PrfA. Importantly, σ^B also contributes to the transcriptional regulation of *prfA* itself, through the *prfA* P2 promoter, one of three *prfA* promoters. Contributions of σ^B to transcription from the *prfA* P2 promoter have been confirmed by phenotypic characterization of deletion mutants (127), as well as by experiments with reporter fusions (128) and *in vitro* transcription assays (129). Interestingly, the *prfA* P2 promoter is not only σ^B dependent but also contains a PrfA binding site. The specific role of this PrfA binding site for regulating *prfA* transcription remains to be clearly defined (129–132). A number of studies have also shown that σ^B regulates the transcription of *inlA* (8, 9, 133) via the σ^B-dependent *inlA* P4 promoter, one of four *inlA* promoters (8, 9, 133). *inlA* encodes internalin A, which is the binding partner of host cell E-cadherin (134). This interaction plays a crucial role in the attachment of *L. monocytogenes* to host cells and their subsequent internalization by the host cells (135). The importance of σ^B-dependent transcription of *inlA* has been demonstrated through guinea pig oral infection experiments, which showed clear virulence attenuation for both a *sigB* null mutant and a mutant with the σ^B-dependent *inlA* P4 promoter deleted (133). The importance of InlA for host cell invasion may explain why a *sigB* mutant is less invasive in CaCo-2 cells, which express high levels of E-cadherin (136), and in guinea pig intestinal infection models but not when the gastrointestinal tract was circumvented by inoculating guinea pigs intravenously (133). This study also indicated a role for σ^B in virulence beyond the upregulation of InlA, as further supported by the fact that a *sigB*

null mutant showed more severe virulence attenuation in a guinea pig oral infection model than a strain carrying only a mutation in the σ^B-dependent *inlA* P4 promoter, indicating a role for σ^B in addition to regulation of *inlA* through the P4 promoter.

Additional important roles for σ^B in fine-tuning gene expression during *L. monocytogenes* infection include indirect repression of the flagellar genes (91) and mediation of reduced expression of the PrfA regulon (126). σ^B-dependent downregulation of flagellar genes (91) occurs through a long 5′ untranslated region (UTR) that is transcribed from a σ^B-dependent *mogR* P1 promoter. This 5′ UTR acts as an antisense RNA to the flagellar genes on the opposite strand. Overexpression of the *mogR* P1 transcript leads to decreases in motility and flagellar gene transcript levels in *L. monocytogenes*. This observation also explains previous findings that *L. monocytogenes sigB* mutants show increased motility at 30°C (13), as the absence of the σ^B-dependent antisense RNA in the 5′ UTR of *mogR* removes one level of repression. Interestingly, Ollinger et al. (126) showed that, in the presence of high levels of active PrfA, σ^B appears to indirectly downregulate the transcript levels of genes in the PrfA regulon, including *hly* and *actA*. These classical, PrfA-dependent *L. monocytogenes* virulence genes play pivotal roles during pathogenesis by enabling bacterial escape from the phagocytic vacuole (*hly*) and spread to neighboring host cells (*actA*). Indirect negative regulation of virulence by σ^B was most prominent in *L. monocytogenes* strains carrying two mutations: a *prfA** mutation (137) that renders the PrfA transcriptional regulator of virulence genes constitutively active, along with a concomitant *sigB* null mutation. σ^B-dependent modulation of PrfA regulon expression reduced the cytotoxic effects of a PrfA* strain in HepG2 cells, possibly facilitating extended intracellular survival and, hence, reduced exposure to extracellular antimicrobial compounds. While this σ^B-dependent downregulation has been shown at both the transcriptional (through microarray hybridization analysis) and the phenotypic (through hemolysis assays) level (126), the mechanism remains to be elucidated. Overall, the modulating role of σ^B in the expression of flagellar, *hly*, and *actA* genes might serve to balance virulence protein levels during the challenging first stages of host infection. For example, downregulation of *hly* may prevent host cell lysis. This hypothesis is consistent with the findings of Glomski et al. (138), who showed that a strain carrying an *hly* allele that is more active than that of the wild type was attenuated in virulence. σ^B-dependent downregulation of flagellar gene expression, on the other hand, may serve to attenuate the host immune response. Overall, σ^B-mediated resilience of *L. monocytogenes* during host infection thus involves many mechanisms, including resistance to key stress conditions encountered by the pathogen. Such resistance can result from direct regulation of a single gene (e.g., *bsh*) or from modulation of regulatory networks, which together facilitate survival at both the extra- and intracellular stages of infection.

Conflicting data exist on the role of σ^B in *S. aureus* virulence, in part because an important experimental strain (*S. aureus* 8325-4) and its derivatives harbor a mutation in *rsbU*, which codes for a key positive regulator of σ^B activity (93). These *rsbU* mutant strains can cause human infections (139), but this mutation explains why early studies performed with these strains (7, 140, 141) found no influence of σ^B on virulence. However, results from *S. aureus* virulence investigations are inconsistent even in strains with an intact σ^B operon. For example, Bischoff et al. (54, 116) and Atwood et al. (114) found that σ^B positively regulates *sarA*, one of the main transcription factors of virulence genes, in *S. aureus* strain Newman (54, 116) and USA300 strains (114). These results were not confirmed by Horsburgh et al. (142), who found no effect of a *sigB* deletion in *S. aureus* SH100 on SarA at either the transcriptional or the translational level and no difference in pathogenicity between the wild-type strain and its isogenic *sigB* mutant in a mouse skin abscess model (142). Other *in vivo* studies found decreased virulence of *sigB* mutants in models of arthritis (in *S. aureus* SH100) (143) and metastatic organ infections (144) but no effects of a *sigB* deletion on pathogenicity in a mouse pyelonephritis and rat osteomyelitis model (*S. aureus* WCUH29) (145). The roles of SarA in these *in vivo* studies cannot be compared because only Horsburgh et al. (142) specifically investigated *sarA* expression levels. It is conceivable that σ^B acts as a modulator of virulence in *S. aureus*, a conclusion that has been put forward by several authors (54, 146, 147) and is supported by a recent study that evaluated the role of σ^B during intracellular growth of *S. aureus* in a host cell line (148). This study showed σ^B-dependent changes in the expression of several genes at the mRNA and protein levels. Specifically, σ^B was found to positively regulate the expression of the transcription factor encoded by *spoVG* and to negatively regulate hemolysin A (*hla*) expression, most likely through σ^B-dependent regulation of genes encoding other transcription factors (139). SpoVG directly regulates a small subregulon that includes genes involved in virulence (149); therefore, σ^B-dependent transcription of *spoVG* might be responsible for indirect σ^B-dependent effects on the transcription of genes that lack a direct σ^B-dependent promoter (55). The mechanism of negative regulation of *hla* by σ^B is unknown. Additionally, σ^B involvement in *S. aureus* virulence appears to occur through σ^B-dependent expression of adhesins, exoproteins, and toxins (54) and through the expression of virulence genes in small-colony variant (SCV) *S. aureus* (150). Several studies showed that the role of σ^B-dependent virulence gene expression is more prominent in SCVs than in the parent strains and that σ^B is involved in the emergence of SCV subpopulations (151–153). In contrast, Bui et al. (154) found a single-nucleotide polymorphism in the *rsbU* gene in a line of *S. aureus* that stably grew as an SCV, which seems to indicate that a strain with largely inactive σ^B is also able to grow with the SCV phenotype. SCVs are associated with chronic infections (for a review, see reference 155), and their emergence appears to result from adaptation to the host environment. The observation that the σ^B regulon diverges between SCVs and the parent strains illustrates the dynamic nature of gene regulation, including possible consequences of selection pressures that may drive modification of regulatory networks to allow for resilience under different conditions.

In *S. aureus*, σ^B indirectly and negatively regulates the virulence factor toxic shock syndrome toxin 1 (TSST-1) (156) and the putative virulence factor hyaluronidase (88). TSST-1 is the causative toxin of the potentially fatal toxic shock syndrome and is encoded by *tst* on a mobile genetic element present in some *S. aureus* strains. Andrey et al. (156) showed that σ^B indirectly represses *tst* transcription via a mechanism involving SarA and Agr. While hyaluronidase provides bacteria access to an abundant carbon source within host tissue (88), it is also speculated to play a direct role in pathogenesis by making host tissue more penetrable, although this notion has not been proven experimentally (157–160). Supporting this hypothesis is the fact that a *hysA* mutant is attenuated in a mouse skin abscess model (86). Incidentally, the

parent strain used in these experiments (*S. aureus* 8325-4) is likely to express increased hyaluronidase activity due to a mutation in *rsbU*, which constitutively suppresses σ^B activity (142, 161), thereby abolishing the indirect negative effect of σ^B on *hysA* transcription.

The increasing evidence supporting roles for σ^B and other transcriptional regulators in bacterial resilience, both in extra- and intrahost environments, also provides an opportunity for discovery of new drugs for the treatment or prevention of infections with Gram-positive pathogens such as *L. monocytogenes* or *S. aureus*. For example, small molecules that interfere with σ^B activation or the assembly of a σ^B-containing RNA polymerase holoenzyme could represent potential therapeutics. The feasibility of identifying such agents is supported by studies that have identified a small molecule that interferes with σ^B activation in *L. monocytogenes* and *B. subtilis* (162), as well as another small molecule that inhibits the binding of the *Escherichia coli* extracellular stress sigma factor σ^E to the polymerase core enzyme (163). Targeting transcriptional regulators that are broadly involved in resilience may also provide an opportunity to develop drugs with reduced risk for the development of resistance mechanisms that become fixed in a pathogen population, as resistance-conferring mutations will likely reduce an organism's resilience in diverse environments.

CONCLUSIONS

While σ^B has been characterized as a stereotypical stress response alternative sigma factor, emerging evidence suggests a much broader role for this alternative sigma factor in resilience, as supported by contributions of σ^B and the σ^B regulon to metabolism, cell envelope homeostasis, and biofilm formation and to pathogen transmission and virulence across different Gram-positive bacteria. σ^B not only facilitates bacterial survival under one or more stress conditions, including during a series of sequential stress exposures, but also provides critical contributions to the regulation of gene expression in complex and rapidly changing environments, such as during host infection or during growth and survival in extrahost environments, including in foods. σ^B appears to facilitate resilience by inducing the expression of genes that allow for survival and growth under subsequently encountered stress conditions. In the case of Gram-positive pathogens, such as *L. monocytogenes*, these connections suggest that the extrahost environment can affect infectivity and virulence; for example, through modulating the expression of stress response and virulence genes that play important roles in the initial phases of the pathogen-host interaction. Additionally, the connection between σ^B and pathogenesis further supports mechanistic links between stress response and virulence and opens up interesting avenues to target sigma factors for drug development (162, 164). Importantly, our emerging recognition of the broad contributions of σ^B to bacterial resilience have been critically aided by the emergence of high-throughput methods (e.g., RNA-seq) and an integrative approach to understanding regulatory networks as a whole, in addition to the study of isolated regulons. Future high-resolution single-cell approaches for characterizing gene expression (e.g., single-cell RNA-seq) will likely provide new insights into the roles of σ^B in bacterial resilience. We hypothesize that stochastic gene expression patterns may generate highly resilient bacterial subpopulations. Future studies will also likely identify additional transcriptional regulators that contribute to bacterial resilience, leading to recognition of resilience as a key theme in bacterial physiology.

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